Research Article

Efficacy of Fungicides, Botanicals and Bio-Agents against *Exserohilum Turcicum*

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Abstract

Maize is one of the important cereal crops of India. Maize is utilized as a staple food by the lower strata of the society and also used as a crop par excellence for industrial use. Among the foliar diseases affecting maize, turcicum leaf blight is of worldwide importance. For managing the foliar diseases use of fungicides is a common practice. So, new fungicides, botanicals and bio agents were screened under *in-vitro* to know its efficacy against *Exserohilum turcicum*. Among the systemic fungicides, tebuconazole completely inhibit the pathogen growth at all the concentrations tested. In contact fungicides, propineb was highly effective as it inhibited the *E. turcicum* up to 83.89 per cent at 500 ppm and among combi products, only Carbendazim 12% + Mancozeb 63% at 500ppm showed complete inhibition of the mycelial growth of *E. turcicum* at higher concentrations. Among the botanicals, garlic bulb extract and in bioagents, *Trichoderma harzianum*- 2 and *Trichoderma viride* showed maximum inhibition of mycelial growth of *E. turcicum*.

Keywords: *Exserohilum turcicum*, turcicum leaf blight, fungicides, botanicals, bioagents

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Introduction

Maize (*Zea mays* L.) is an important food and feed crop which ranks third after wheat and rice in India and the world. Because of its expanded use in the agro-industries it is recognized as a leading commercial cereal crop of great agro-economic value [9]. Among cereal crops, maize has the highest average yield per ha and stands third after wheat and rice in total area and production in the world [5]. Maize ranks first in world production (960 million tonnes) followed by wheat (691 million tonnes) and rice (461 million tonnes) [3]. This represents 38 per cent of the total grain production from maize as compared to 30 per cent for wheat and 20 per cent for rice. United States is the largest maize producer followed by Brazil, Ukraine and Argentina. During the last few years, there has been a progressive escalation in demand for maize grain for the value added products like glucose, sorbitol, dextrose and oils, besides livestock, poultry and animal feeds; it is also used for manufacture of starch and starch based products. In Indian agriculture, maize occupies an important place after wheat and rice. Maize is not only utilized as a staple food by the lower strata of the society, but it is also used as a crop par excellence for industrial use. Maize is cultivated under diverse environmental conditions. Among the cereals, in India, maize occupies fifth place in area, third place with respect to production and productivity. India grows about 8.71 million hectares of maize with total annual production of 22.3 million tonnes of grain giving an average yield of 2.55 tonnes per hectare [3] which ranks third in production and contributes to 2.4 per cent of world production with almost 5 per cent share in world harvested area in 2013-14. In India, Karnataka, Andhra Pradesh, Maharashtra, Tamil Nadu, Rajasthan and Uttar Pradesh together contribute to 60 per cent of area and 70 per cent of maize production. Karnataka is one of the major maize producing states in the country. During the cropping year of 2012-13, maize was grown over an area of 11.26 lakh hectares with a production of 34.00 lakh tonnes. The productivity of the state is 36.50 quintals per ha [2] closely followed by Andhra Pradesh (29.90 q/ha). As the maize cultivation reaches its boom in terms of acreage, adoption of modern crop production practices like, using chemical fertilizers, chemical pesticides and also use of some commercial hybrids. These factors in several ways led the maize crop could vulnerable to pests and diseases at the farmers field. Among the different diseases, foliar diseases are of significant importance. These foliar diseases destroy the leaves and result in significant yield reduction. Among the foliar diseases affecting maize, the Turcicum leaf blight also called Northern corn leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. (syn. *Helminthosporium turcicum* Pass.) is of worldwide importance. Turcicum leaf blight is one of the most important fungal diseases affecting photosynthesis with severe reduction in grain yield of more than 50 per cent [13, 12]. The disease is more prevalent in Andhra

Pradesh, Karnataka, Bihar, Himachal Pradesh and Maharashtra. Turcicum leaf blight is considered to be one of the most devastating foliar diseases in Karnataka resulting in reduction of grain yield by 28 to 91 per cent [8, 11].

Use of fungicides for the control of plant diseases is a common practice. As *Exserohilum* is a air borne pathogen and developed resistant to many of the fungicides. So we have to look for newer fungicides, botanicals and bio-agents to use these in integrated management practices, hence studies were undertaken to evaluate new fungicides, botanicals and bio-agents to know their efficacy against *Exserohilum turcicum* under lab conditions for further utilization in field to manage the disease.

Experimental methods

Evaluation of fungicides against Exserohilum turcicum

The efficacy of six systemic fungicides (at the concentrations of 100, 150, 200, 250 ppm), three non systemic fungicides (at the concentration of 100, 200, 300, 500 ppm) and four combi products (at the concentration of 100, 200, 300, 500 ppm) were assayed against *Exserohilum turcicum.* These fungicides were evaluated in laboratory conditions by following "Poison food technique" [6].

Poison food technique

Required quantity of individual fungicide was prepared and added separately in to sterilized molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates. Mycelial discs of five mm size from seven days old culture was cut by a sterile cork borer and one such disc was placed at the centre of each agar plate. The plate without any fungicide served as control. Three replications were maintained for each concentration. Such plates were incubated at room temperature and the radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the fungicides was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the formula given by Vincent (1947) [17].

$$I = \frac{C - T}{C} \times 100$$

Where, I = per cent inhibition, C = growth in control, T = growth in treatment

Evaluation of botanicals

The efficacy of eleven botanical extracts was assayed against *Exserohilum turcicum.*

Preparation of botanicals

Fresh leaves/ bulbs of plants were collected from various locations of UAHS, Shivamogga and their taxonomical identification confirmed. These samples were washed thoroughly with tap water and surface sterilized with 0.1 per cent sodium hypochlorite and repeatedly washed with distilled water. Hundred grams of leaf/bulb materials were taken and cut in to small pieces, 100 ml water was added and the leaf/bulb materials were heated and crushed using a grinder. Ground stock solution of all the leaf extracts was collected by filtering with muslin cloth. These botanicals were evaluated in laboratory conditions by following "Poison food technique" [6].

Poison food technique

Required quantity of individual botanical was added separately into sterilized molten and cooled potato dextrose agar so as to get the desired concentration of the botanical and the remaining procedures was same as explained under evaluation of fungicides.

Evaluation of Bio-agents

In vitro evaluation was carried out with five bio-agents against *Exserohilum turcicum* through dual culture technique. For this study both fungal bioagents and test fungus were cultured on potato dextrose agar and bacterial bioagents were cultured on nutrient agar in order to get fresh and active growth of fungus and bacteria.

Dual culture technique

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus and antagonistic fungus were placed opposite to each other of the Petri plate. In case of evaluation of bacterial antagonist, the bacterium was streaked one day earlier at middle of the Petri plate and the test fungus placed at both the ends. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947) [17].

Results and Discussion

Evaluation of fungicides against Exserohilum turcicum

Systemic fungicides

The per cent inhibition of mycelial growth of *E. turcicum* in different systemic fungicides was calculated. Among systemic fungicides, tebuconazole was found to be highly effective at all concentrations tested as it inhibited cent per cent growth of *E. turcicum* at all the concentrations tested and was significantly superior over control and other fungicides. Among the other tested systemic fungicides propiconazole was found to be effective as it inhibited the growth of the *E. turcicum* up to 70 per cent at the higher concentration tested. Carbendazim, hexaconazole, difenconazole and azoxystrobin also showed the inhibition in the mycelial growth of *E. turcicum* up to 65.83%, 64.44%, 63.33% and 62.22% respectively at higher concentrations, but not up to the tebuconazole and propiconazole fungicides (**Table 1** and **Figure 1**). These results were in accordance with the results of Harlapur *et al.* (2007) [7], who reported that propiconazole was found to inhibit the growth of *E. turcicum* and in present study tebuconazole found to be more effective. It is because tebuconazole also belongs to triazole group of fungicides and these triazole fungicides inhibit specific enzyme C_{14}-demethylase, which plays a role in sterol production. These sterols are needed for membrane structure and function, making them essential for the development of functional cell walls.

Table 1 Efficacy of systemic fungicides against *Exserohilum turcicum*

Fungicides	**% Inhibition**				
	100 ppm	**150 ppm**	**200 ppm**	**250 ppm**	**Mean**
Hexaconazole	44.44 (41.81)*	57.39 (49.25)	59.17 (50.28)	64.44 (53.47)	56.36
Propiconazole	62.78 (52.41)	63.61 (52.91)	66.67 (54.75)	70.56 (57.15)	65.90
Difenconazole	59.44 (50.44)	60.28 (50.93)	63.33 (52.75)	63.33 (52.73)	61.60
Azoxystrobin	18.33 (25.15)	28.89 (32.08)	56.94 (49.00)	62.22 (52.07)	41.60
Carbendazim	63.61 (52.90)	63.61 (52.89)	65.56 (54.06)	65.83 (54.23)	64.65
Tebuconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
Mean	49.80	53.40	58.81	60.91	
	SE m±		CD at 1 %		
Fungicides (F)	0.94		2.73		
Concentration(C)	0.71		2.07		
F X C	1.88		5.46		

*figures in parenthesis are arc sine transformed values

Non-systemic fungicides

Among the non systemic fungicides, propineb was highly effective at higher concentration as it inhibited the *E. turcicum* up to 76.94, 81.94, 82.22 and 83.89 per cent at 100, 200, 300 and 500 ppm respectively. Captan also found to be effective in inhibiting the mycelial growth up to 81.94 per cent at 500 ppm concentration. Mancozeb was found to be less effective in inhibiting the growth of *Exserohilum turcicum* compared to other two contact fungicides as it inhibited mycelium growth up to 78.06 per cent at 500 ppm concentration (**Table 2**). Many workers like Harlapur *et al.* (2007) [7] and Wathaneeyawech *et al.* (2015) [18] reported that Mancozeb was effective in inhibiting the growth of the fungus but, the present investigation results were contrary to this which may be due to the variation in the

isolates and due to the injudicious use of this mancozeb over a long period of time which may result in development of resistant to the particular fungicides by the pathogen.

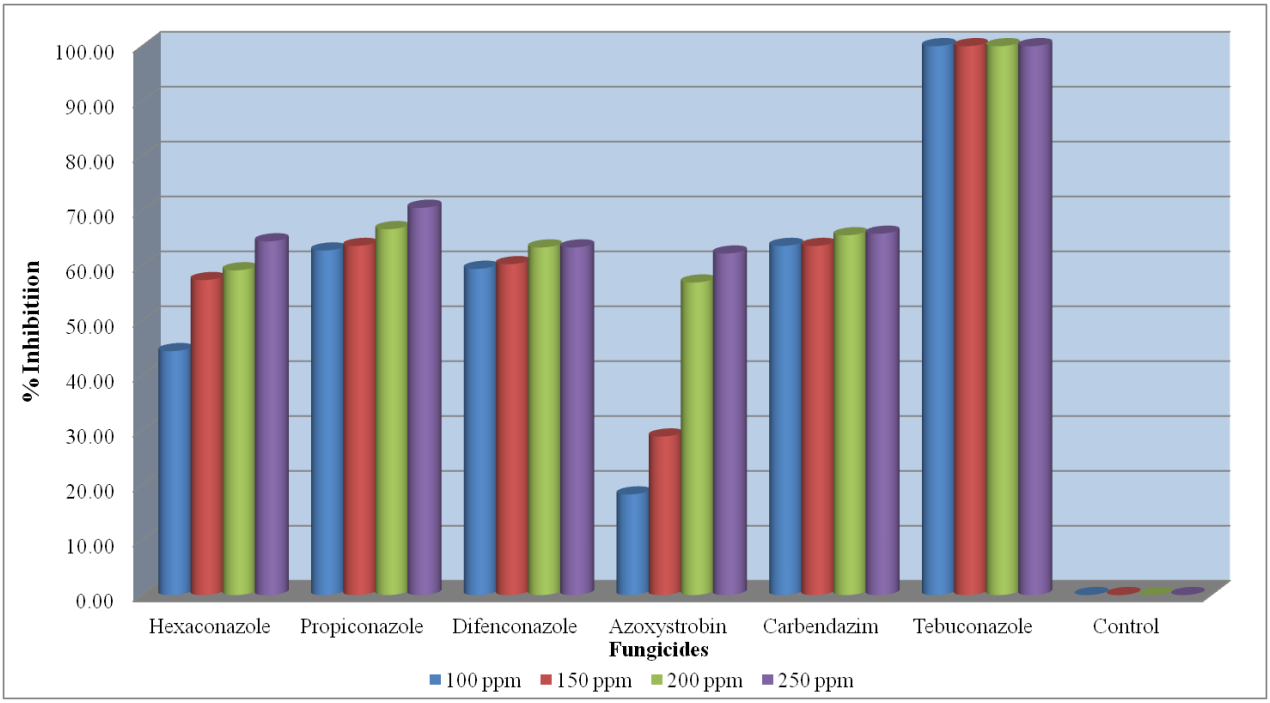

Figure 1 Efficacy of systemic fungicides on *Exserohilum turcicum* under *in vitro* conditions

Table 2 Efficacy of non-systemic fungicides against *Exserohilum turcicum*

Fungicides	**% Inhibition**				
	100ppm	**200ppm**	**300ppm**	**500ppm**	**Mean**
Captan	77.78(61.85)*	79.17(62.83)	81.11(64.22)	81.94 (64.82)	80.00
Propineb	76.94(61.28)	81.94(64.82)	82.22(65.03)	83.89 (66.30)	81.25
Mancozeb	71.39(57.64)	74.17(59.43)	75.56(60.34)	78.06 (62.04)	74.79
Control	0.00(0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
Mean	56.53	58.82	59.72	60.97	
		SE m±	CD at 1 %		
Fungicides (F)		0.24	0.73		
Concentration (C)		0.24	0.73		
F X C		0.48	1.46		
*figures in parenthesis are arc sine transformed values					

Combi products

All the combi-products were effective in inhibiting the mycelial growth of the *E. turcicum* at all the four concentration tested. Among these only Carbendazim 12% + Mancozeb 63% at 500ppm showed complete inhibition of the mycelial growth of *E. turcicum* and other showed up to maximum of 89.72 % inhibition. Tebuconazole 50% + Trifloxystrobin 25% was able to inhibit the mycelial growth up to 86.39, 88.89, 89.17 and 89.72 per cent at 100, 200, 300 and 500ppm concentrations. The inhibition of mycelial growth was 87.78 % at 500ppm concentrations in Carbendazim 25% + Iprodione 25% fungicide, whereas Metalaxyl 4 % + Mancozeb 68 % usually recommended for oomycete fungi also showed mycelial inhibition up to 84.44 at 500ppm concentrations under *in vitro* conditions (**Table 3**).

The effect of combi products were reported by Sanjeev Kumar and Mauriya (2015) [14]; Veerabhadraswamy *et al.* (2014) [16]; Anand *et al.* (2013) [1] they found that Carbendazim 12% + Mancozeb 63% and Tebuconazole 50 % + Trifloxystrobin 25 % were found to inhibit the growth of the fungus at several concentrations. It is due to the action of these fungicides on fungal cell as they disrupt the membrane function of the cell wall by inhibiting the C_{14} demethylase enzyme and the contact fungicides interfering with the oxygen uptake and inhibit the suphahydral system of the fungus.

Table 3 Efficacy of combi-product fungicides against *Exserohilum turcicum*

Fungicides	% Inhibition				
	100ppm	200ppm	300ppm	500ppm	Mean
Tebuconazole 50% + Trifloxystrobin 25%	86.39(68.36)*	88.89(70.53)	89.17(70.80)	89.72(71.31)	88.54
Carbendazim 25% + Iprodione 25%	86.94(68.82)	87.22(69.06)	87.78(69.54)	87.78(69.55)	87.43
Carbendazim 12% + Mancozeb 63%	83.06(65.69)	83.61(66.12)	85.28(67.44)	100.00(90.00)	87.99
Metalaxyl 8 % + Mancozeb 64 %	76.11(60.76)	79.72(63.23)	84.44(66.77)	84.44(66.78)	81.18
Control	0.00(0.00)	0.00 (0.00)	0.00 (0.00)	0.00(0.00)	0.00
Mean	66.50	67.89	69.33	72.39	
	SE m±		CD at 1 %		
Fungicides (F)	0.30		0.89		
Concentration (C)	0.27		0.79		
F X C	0.60		1.77		

*figures in parenthesis are arc sine transformed values

Evaluation of Botanicals

The experiment was conducted to assess the antifungal activity of eleven plant extracts at three different concentrations. The effect of plant extracts on the per cent inhibition of mycelial growth of *E. turcicum* at three concentrations differs significantly and it is observed that the increasing the concentration of botanicals increases efficacy against the *E. turcicum.* Thus at 10 per cent, cent percent inhibition of mycelial growth was observed in Garlic bulb extract which was significantly superior over other plant extract followed by Parthenium leaf extract (70.00 %), Ginger extract (63.15 %), onion bulb extract (57.41 %), pongamia leaf extract (51.11 %), Neem leaf extract (49.26 %), Nilgiri leaf extract (45.00 %) and Noni leaf extract (42.96 %). Least inhibition of mycelial growth was observed in Ocimum leaf extract (9.44 %) followed by leucas leaf extract (20.74 %) and Agarwood leaf extract (29.07 %).

Among the 11 plant extracts, mean maximum per cent inhibition of mycelial growth (79.63 %) was recorded in Garlic bulb extract which was significantly superior over all other botanicals tested, followed by parthenium leaf extract (64.14 %), Pongemia leaf extract (48.89 %), Ginger extract (46.79 %), Neem leaf extract (43.33 %) and Onion bulb extract (40.72 %). Whereas, least per cent inhibition was recorded in Ocimum leaf extract (7.59 %), Leucas leaf extract (12.78 %), Agarwood leaf extract (17.22 %) and Noni leaf extract (30.19 %) (**Table 4** and **Figure 2**).

Table 4 Efficacy of botanicals against *Exserohilum turcicum*

Sl. No.	Concentration	% inhibition			
		2.5%	5%	10%	Mean
Botanicals					
1	*Ocimum sanctum*	5.93	7.41	9.44	7.59
2	*Leucas aspera*	7.41	10.19	20.74	12.78
3	*Eucalyptus globules*	24.07	32.78	45.00	33.95
4	*Morinda citrifolia*	12.04	35.56	42.96	30.19
5	*Aquilaria agallocha*	0.00	22.59	29.07	17.22
6	*Zingiber officinale*	38.33	38.89	63.15	46.79
7	*Allium cepa*	22.78	41.67	57.41	40.62
8	*Allium sativum*	64.81	74.07	100.00	79.63
9	*Parthenium hysterophorus*	60.74	61.67	70.00	64.14
10	*Pongamia pinnata*	45.37	50.19	51.11	48.89
11	*Azadirachta indica*	36.85	43.89	49.26	43.33
12	Control	0.00	0.00	0.00	0.00
Mean		26.53	34.91	44.85	
		SE m ±		CD at 1 %	
Botanicals (B)		0.27		0.77	
Concentration (C)		0.14		0.38	
B X C		0.47		1.32	

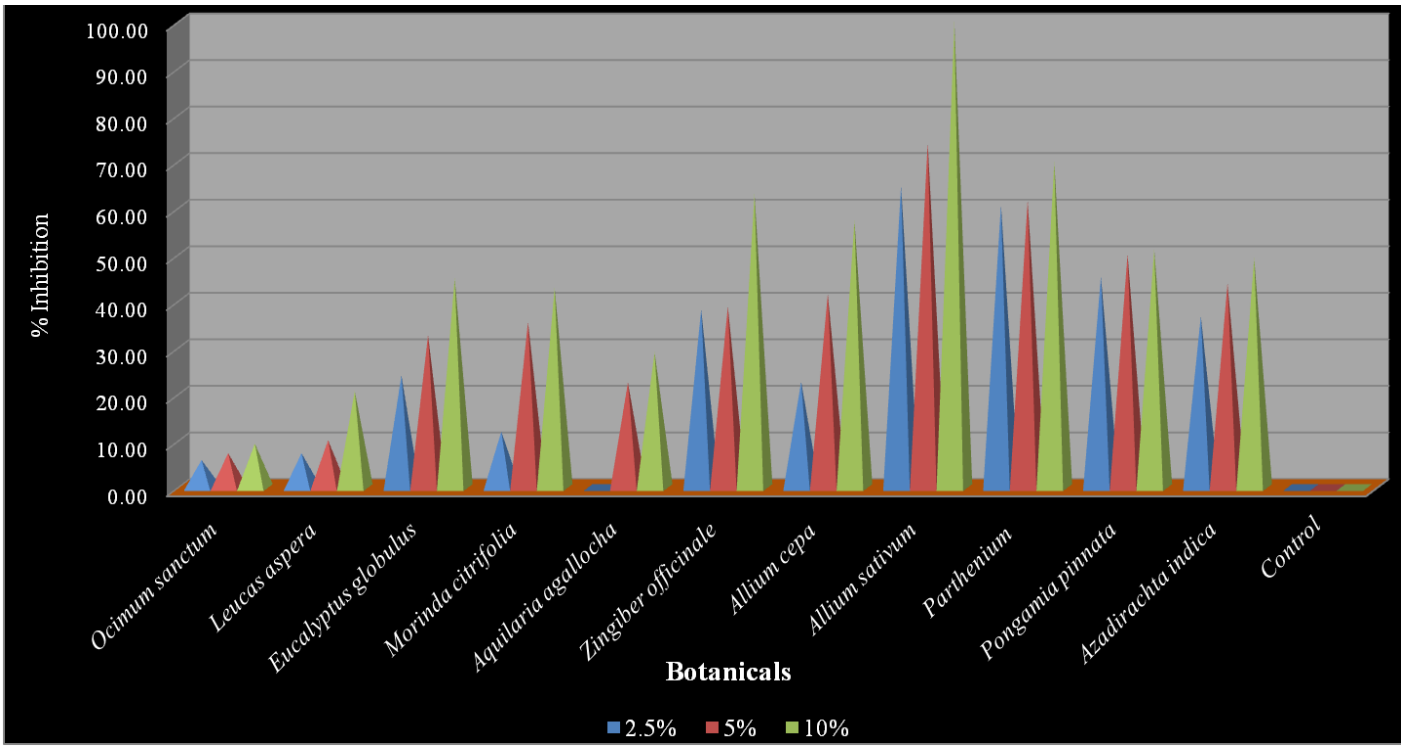

Figure 2 Effect of botanicals on the mycelial growth of *Exserohilum turcicum*

Several authors tested different botanicals and showed botanicals were effective in inhibiting the mycelial growth. Bhati *et al.* (2011) [4] reported that *Crinum latifolium* showed 72.20 per cent mycelial growth inhibition followed by *Terminalia chebula* (66.60%). Harlapur *et al.*, 2007 [7] reported that, neem seed kernel extract @ 5 per cent concentration caused maximum inhibition of growth (56.64%) followed by *Aloe vera* @ 10 per cent (53.50%) and Khedekar *et al.* (2012) [10] reported that nimbicidin with 71.27 per cent inhibition of mycelial growth was most effective among all the plant extracts. In this present investigation also neem leaf extract showed up to 43 per cent inhibition. In this study inhibition of mycelial growth of fungus by garlic is may be due to the presence of antibiotic allicin in garlic extract and parthenium leaf extract also found to inhibit the mycelial growth. So, we can use the noxious weed parthenium against this pathogen so that we can manage both parthenium and *E. turcicum.*

Table 5 Efficacy of bio-agents against *Exserohilum turcicum*

Sl. No.	Bio- agent	% Inhibition
1	*Trichoderma harzianum* Rifai – 1	85.37 (71.36)*
2	*Trichoderma harzianum* Rifai – 2	98.65 (83.27)
3	*Trichoderma viride Pers.* Ex. S. F. Gray	98.34 (84.07)
4	*Pseudomonas fluorescens* Migula – 1	95.49 (77.99)
5	*Pseudomonas fluorescens* Migula – 2	94.24 (76.11)
6	Control	0.00 (0.00)
	SE m±	4.02
	C.D at 1%	12.84

*figures in parenthesis are arc sine transformed values

Evaluation of bio-agents

The antagonistic microorganism's *viz.*, *Trichoderma harzianum* Rifai-1, *Trichoderma harzianum*-2, *Trichoderma viride* Pers. Ex. S. F. Gray, *Pseudomonas fluorescens* Migula- 1 and *Pseudomonas fluorescens* Migula- 2 were evaluated by dual culture technique for their antagonistic effect against *Exserohilum turcicum* under *in-vitro* conditions. Maximum inhibition of mycelial growth (98.65%) was noticed in *Trichoderma harzianum* -2, which was

followed by *Trichoderma viride* (98.34%). Among the bacterial antagonists, *Pseudomonas fluorescens* -1 and *Pseudomonas fluorescens- 2* showed the mycelial inhibition of 95.49% and 94.24% respectively. Among the five bio agents tested, least mycelial growth inhibition was observed in *Trichoderma harzianum* Rifai-1 (85.37%) (**Table 5**). These results were in accordance with the results of Harlapur *et al.* (2007) [7] and Khedekar *et al.* (2012) [10] who reported that *Trichoderma harzianum* was effective in inhibiting the mycelial growth. These results are further supported by the results of Singh and Singh (2014) [15], who reported that both *Trichoderma harzianum* and *Pseudomonas fluorescens* were effective in inhibiting the growth of the fungus.

Conclusions

Fungicides, tebuconazole, propineb and Carbendazim 12% + Mancozeb 63% were effective in inhibition of *E. turcicum.* Among the botanicals, garlic bulb extract and the bioagents, *Trichoderma harzianum*- 2 and *Trichoderma viride* showed maximum efficacy against *E. turcicum.* These fungicides, botanicals and bio-agents should be tested under field conditions to confirm its efficacy.

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